Pretreatments of Wheat Straw for Separation into Major Components

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Summary

Pretreatments removed various amounts of hemicellulose and lignin from wheat straw (WS) and upgraded the cellulosic residue for enzymatic conversion to glucose. Five pretreatments performed on WS involved thermal hydropulping, dilute acid pulping, thermal hydropulping followed by alkali extraction, alkali extraction, and alkaline hydrogen peroxide. Dilute acid pulping of WS provided xylose in the liquor, whereas liquors from other treatments contained xylan. Cellulose contents were increased from 33% in the WS to 45–65% in pulps that were attained in 52–71% yield. Treatment of the pulp residues with cellulase converted 27–55% of the pulps (58–98% pulp cellulose converted) to glucose. Alkaline hydrogen peroxide pretreatment provided the highest yield of glucose.

INTRODUCTION

Utilization of biomass for chemicals and/or fuels requires improved technology that provides substrates more amenable to hydrolysis. Multistage processes to separate the major components from lignocellulosic materials have been offered as a solution to problems associated with biomass utilization [1]. Extensive studies have provided techniques for the conversion of resistant raw materials to substrates more susceptible to hydrolysis [2]. The effects of biological, chemical, physical, and thermal pretreatments on the accessibility of cellulose to enzymatic hydrolysis have been investigated [3–7]. Practical commercial pretreatments should provide more than a physical disruption of plant cell structure [8]. Such pretreatments should use simple and inexpensive chemicals, equipment, and techniques; solubilize lignin and hemicellulose if pentoses are desired; enhance the hydrolysis of cellulose, and be adaptable to many species of plants.

The present investigation into pretreatments of wheat straw (WS) permits a comparison of processes to separate and recover the major components.

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MATERIALS AND METHODS

Baled straw of soft winter wheat, *Triticum aestivum*, variety Arthur, from central Illinois was cut into 2 to 8 cm lengths in a Taylor, Stiles, and Company chopper. The longer sections were cut manually to 2.5 cm. Five methods were used to modify WS.

Thermal Hydropulping

Chopped straw (135 g, moisture-free basis) and water (945 g) were sealed in a 2 L autoclave and rocked in an electric heating unit. A predetermined temperature in the range of 180 to 185°C was reached in about 50 min and maintained for 30 min. The autoclave was removed from the heating unit, allowed to cool until pressure reached 120 psig, and then cooled with ice until the internal pressure reached zero (20 min). The contents were filtered on a stainless steel funnel, and pulps were washed four times with hot water (600 mL, total). Pulp and liquor samples were freeze dried and then analyzed.

Dilute Acid Pulping

Samples of chopped straw were pulped in the 2 L autoclave with 1, 2, or 3% H_2SO_4 (7, 14, or 21% based on dry straw) with a liquid-to-solid ratio of 7:1. Pulping was for 1 h at 130°C after a 30 min warmup. The autoclave was then cooled with ice until the internal pressure was zero (10 min). The contents of the autoclave were filtered and washed as described above. Pulp samples were freeze dried before analyses. A sample of the liquor was neutralized with calcium hydroxide and sodium carbonate before being analyzed for xylose by high-performance liquid chromatography (HPLC).

Alkali Extraction after Thermal Hydropulping

The thermal hydropulping for this experiment was performed at 180°C for 30 min. The derived pulp was extracted with NaOH (8%, based on pulp solids) for 30 min at 70°C and 3% consistency. Samples were filtered, washed, and freeze dried as previously described. The pH of filtrates was adjusted to 4.5 with CH_3COOH. Precipitates, formed at pH 4.5, were freeze dried. To the supernatants, 95% ethanol was added to give a 2:1 (ethanol:water) ratio. Since less than 0.1% precipitate (WS basis) was formed, it was not analyzed.

Alkali Extraction

Chopped WS was ground in a Wiley mill equipped with a screen containing 1 mm diameter openings. On a moisture-free basis, 80 and 40 g WS were mixed with 800 mL 4% NaOH (w/v) and 400 mL 10% NaOH (w/v), respectively. Samples were steeped overnight at room temperature and filtered. Pulps were washed with hot water. The combined filtrates and washes were adjusted to pH 4.5 with CH_3COOH. Diluted filtrates were centrifuged, and the isolated solids

were freeze dried for analyses. To the supernatants, 95% ethanol was added to give an ethanol:supernatant ratio of 2:1, and the mixture was allowed to stand overnight. The precipitates that formed were isolated, treated with 5% H_2SO_4 for 3 h at 98°C, and analyzed for xylose by HPLC. Precipitates from the 10% NaOH extraction were washed with absolute alcohol and air dried before chemical analyses.

Alkaline Hydrogen Peroxide

First, 500 mL 1% H_2O_2 was added to chopped WS (10 g, moisture-free basis) in Erlenmeyer flasks. Then the pH was adjusted to 11.5 before placing the flasks on a Lab-Line Junior Orbit shaker operating at 200 rpm. After 24 h of shaking, samples were filtered, and pulps were washed three times with water (450 mL, total). Liquors were neutralized with CH_3COOH, and pulps and liquors were freeze dried and analyzed.

Substrates and Liquors

A cellulase treatment described by Detroy et al. [3] was used to determine percent hydrolysis of the cellulosic pulp, which had been kept frozen but never dried. Glucose, formed in the cellulase hydrolysis, was determined by HPLC. Cellulose content was determined by a monoethanolamine (MEA) method [9] and reported on an ash- and pentosan-free basis. Lignin analyses were made by the spectrophotometric method of Bagby et al. [10]. Other analytical procedures were standards of the Technical Association of the Pulp and Paper Industry.

RESULTS AND DISCUSSION

Five experimental procedures were evaluated to determine their effectiveness in disrupting the lignin–hemicellulose–cellulose complex and providing fractions rich in a specific component or components. Dilute acid treatment yielded xylose in the liquor, whereas the other treatments gave xylan-rich fractions.

Thermal hydropulping of WS at 180–185°C gave pulp yields of about 70% (Table I). A threefold increase in alcohol–benzene extractives resulted. Compared to the original WS, nearly two to three times as much of the pulp was soluble in alcohol–benzene, depending upon pulping temperature. Essentially all of the cellulose of the WS is accounted for in the pulps, and most WS lignin is accounted for in the pulps and liquors. For hydropulping at 180°C, pentosans in the pulp and liquor were 75% of the original WS pentosans compared to only 47% for hydropulping at 185°C. Not only were the cellulose contents of the pulps high (47–49%), but the cellulose in the pulps was seven to eight times more susceptible to enzymatic hydrolysis than that in chopped WS.

The highest yield of liquor solids was obtained at 180°C, and these solids contained the highest percentage of pentosans, compared to solids recovered

TABLE I

Composition and Properties of Thermal Hydropulped Wheat Straw[a]

Analyses (%)	Pulping temperature (°C)			
	unpulped	180	183	185
	Wheat straw		Pulp	
Yield[b]	–	70.8	69.8	70.1
Solubility in alcohol–benzene	6.5	16.0	22.3	22.7
Cellulose				
MEA[c]	32.9	46.9	46.6	49.0
alpha	30.2	42.1	40.6	42.7
Pentosans	29.1	15.7	10.1	9.0
Lignin	14.5	14.5	10.8	12.7
Ash	9.5	7.2	8.0	7.8
Cellulose conversion	10.0	72.8	79.8	80.9
			Liquor solids	
pH[d]		3.8	3.8	3.8
Yield[b]		22.2	16.0	16.4
Pentosans		48.5	44.9	44.6
Lignin		12.9	21.2	23.0
Ash		16.9	19.2	19.1

[a]Water:straw (2.5 cm), 7:1, 30 min at temperature.
[b]Original wheat straw, moisture-free basis.
[c]Monoethanolamine method [9].
[d]pH of liquor at end of cook.

TABLE II

Composition and Properties of Dilute Acid Pulped Wheat Straw[a]

Analyses (%)	Concentration of H_2SO_4 (%)			
	unpulped	1	2	3
	Wheat straw		Pulp	
Yield[b]	–	67.9	63.0	59.2
Solubility in alcohol–benzene	6.5	17.3	17.4	15.2
Cellulose				
MEA[c]	32.9	46.7	47.3	45.0
alpha	30.2	39.1	25.6	41.6
Pentosans	29.1	14.4	12.0	7.3
Lignin	14.5	13.4	14.2	15.4
Ash	9.5	7.5	7.4	9.5
Cellulose conversion	10.0	57.8	71.0	85.6
			Liquor properties	
pH[d]		1.6	1.3	1.2
Yield of xylose[b]		14.4	15.1	17.4

[a]H_2SO_4 solution: straw (2.5 cm), 7:1, 1 h at 130°C.
[b]Original wheat straw, moisture-free basis.
[c]Monoethanolamine method [9].
[d]pH of liquor at end of cook.

from higher temperature cooks. As expected, the ash contents were high because minerals removed from WS were concentrated in these liquor solids.

Pulp yields were 59–68% from dilute acid pulping of WS (Table II). As with the thermal hydropulps, the alcohol–benzene solubility of the pulps was high. On the basis of original WS, alcohol–benzene extractives from the pulps were increased 180, 169, and 138% after treatments with 1, 2, and 3% H_2SO_4, respectively. Cellulose contents of the pulps were high (45–47%); however, compared to the WS, these values represent from 4 to 19% loss in cellulose during H_2SO_4 treatment (Table II). Susceptibility of the pulp cellulose to enzymatic hydrolysis increased from 58% conversion when WS was pulped with 1% H_2SO_4 to 71 and 86% when pulped with 2 and 3% H_2SO_4. Pentosans in the pulps account for only 15 to 34% of the original WS pentosans. The pulps contained about the same percentage of lignin as the original WS (13–15 vs 14.5%), but these values, as a percentage of the pulps, indicate that 37–39% of the original WS lignin had been removed. Of the minerals in WS, 40 to 50% were removed during acid pulping. The final pH values for these liquors were one to two compared to pH 4 for the liquors obtained from thermal hydropulping. Xylose yields in the liquor hydrolyzate were 14–17%, representing 13–15% xylan.

When autohydrolyzed aspen was extracted with caustic, significant quantities of lignin became soluble according to Lora and Wayman [11]. Compositions of the thermal hydropulp (180°C for 30 min) before and after extraction with 8% NaOH (based on pulp solids) are reported in Table III. Alkali removed an additional 20% of the original WS solids. This resulted in doubling the concentration of cellulose in the WS pulp (65 vs 33%), indicating no loss in cellulose.

TABLE III

Characteristics of Thermal Hydropulped Wheat Straw after Alkali Extraction

Analyses (%)	Straw	Pulp[a]	
		untreated	alkali extracted[b]
Yield[c]	–	71.2	51.6
Cellulose (MEA)[d]	32.9	47.4	65.4
Pentosans	29.1	16.2	12.5
Lignin	14.5	12.4	11.4
Cellulose conversion	10.0	74.8	77.9
		Liquor solids	
pH[e]		4.0	10.6
Yield[c]		22.8	7.8[f]
Pentosans		47.5	6.0
Lignin		12.0	62.6

[a]Water:straw (2.5 cm), 7:1; 30 min at 180°C.

[b]Sodium hydroxide (8%, based on pulp solids, moisture-free), 3% pulp consistency, 30 min at 70°C.

[c]Original wheat straw, moisture-free basis.

[d]Monoethanolamine method [9].

[e]pH of liquor at end of treatment.

[f]Solids precipitated by addition of acetic acid to filtrate.

TABLE IV
Characteristics of Alkali-Extracted Wheat Straw[a]

Analyses (%)	Concentration of NaOH (%)		
	untreated	4	10
Yield[b]	–	66.0	57.3
Solubility in alcohol–benzene	6.5	3.7	5.3
Cellulose			
MEA[c]	32.9	49.0	53.8
alpha	30.2	46.7	48.5
Pentosans	29.1	20.5	12.6
Lignin	14.5	8.2	8.7
Ash	9.5	13.7	17.8
Cellulose conversion	35.0	82.8	91.2
		Precipitated liquor solids	
Yield[d]		23.7 (4.0)	25.9 (8.7)
Pentosans		40.7 (20.7)[e]	51.7 (61.2)
Lignin		16.7	18.5 (6.3)
Ash		21.2	17.4 (12.2)

[a]NaOH solution: straw (−1 mm), 10:1; 24 h at room temperature.
[b]Original wheat straw, moisture-free basis.
[c]Monoethanolamine method [9].
[d]Solids precipitated by addition of acetic acid to filtrates. Values in parentheses represent solids precipitated by the addition of ethanol to the supernatants. Percent on original wheat straw.
[e]Calculated as xylose times 0.88.

This extraction removed 18% more of the pentosans and 20% more of the lignin than the hydropulping of WS alone. The acid-precipitated solids from this extraction were 63% lignin, representing 34% of WS lignin. However, only a slight improvement in cellulose conversion was noted upon enzymatic hydrolysis.

When milled Ws was extracted with 4 and 10% NaOH, pulp yields of 66 and 57%, respectively, were realized (Table IV). Unlike the thermal hydropulping and dilute acid pulping, 63 and 54% of the alcohol–benzene extractives were removed from the WS. Cellulose contents of the residues were 49 and 54%, which account for nearly all of the WS cellulose. These modified substrates were very susceptible to enzymatic hydrolysis, converting 83 to 91% of the cellulose to glucose. Extraction with 10% NaOH removed 75% of the pentosans and 66% of the lignin from WS. Solids that precipitated from the liquor by addition of acetic acid (pH 4.5) were 52% pentosans, and those precipitated by alcohol were 61% pentosans. These combined precipitates contained 64% of the original WS pentosans. As anticipated, the more concentrated alkali was more effective in removing components and in providing a substrate more susceptible to enzymatic attack.

When WS is treated with H_2O_2 as reported by Gould [12], essentially all of the cellulose in the pulp was converted into glucose by cellulase (Table V). Yield of pulp from the treatment was 59%. Alcohol–benzene extractives from

TABLE V

Characteristics of Alkaline Hydrogen Peroxide-Treated Wheat Straw[a]

Analyses (%)	Treatment	
	untreated	1% H_2O_2
Yield[b]	–	59.1
Solubility in alcohol–benzene	6.5	1.5
Cellulose		
MEA[c]	32.9	56.9
alpha	30.2	52.9
Pentosans	29.1	29.5
Lignin	14.5	9.1
Ash	9.5	3.1
Cellulose conversion	10.0	97.5
		Liquor solids
Yield[b]		88.2[d]
Pentosans		12.7
Lignin		11.2
Ash		45.4

[a]H_2O_2 solution: straw (2.5 cm), 50:1, 24 h at room temperature. Shaken at 200 rpm.

[b]Original wheat straw, moisture-free basis.

[c]Monoethanolamine method [9].

[d]Liquor was neutralized with acetic acid before freeze drying. If corrected for ash, value becomes 48.2%.

the pulp were less than 1% (WS basis). With removal of 40% of the pentosans and 63% of the lignin from the WS, the cellulose content of the pulp was 57%. Pulp mineral content was less than 2% (WS basis). Recovery of the pentosans and lignin removed from WS by alkaline hydrogen peroxide treatment was excellent.

Figure 1 provides a comparison of the five pretreatments as to the residual pentosan and lignin contents in the pulps and susceptibility of these cellulose substrates to enzymatic hydrolysis. The residual components are expressed on the basis of the chemical composition of the original WS. The two-step process was the most effective in removing pentosans. For a single step, the NaOH and H_2SO_4 treatments were equally effective in removing pentosans. For lignin removal, the NaOH and alkaline H_2O_2 treatments were the most effective but were similar in effectiveness to the two-step method.

Hydrogen peroxide treatment permitted the best material balances for the pentosans (Fig. 2). However, the greatest quantity of pentosans was recovered in the NaOH liquor. Of the WS pentosans, 89% was present in the pulp and liquor after a 10% NaOH treatment of the WS. When xylose in the liquor from the 2% H_2SO_4 cook is calculated as pentosans, more pentosans were recovered from the acid cook than from the 180°C water cook. However, the total pentosans present in the combined pulps and liquors were similar.

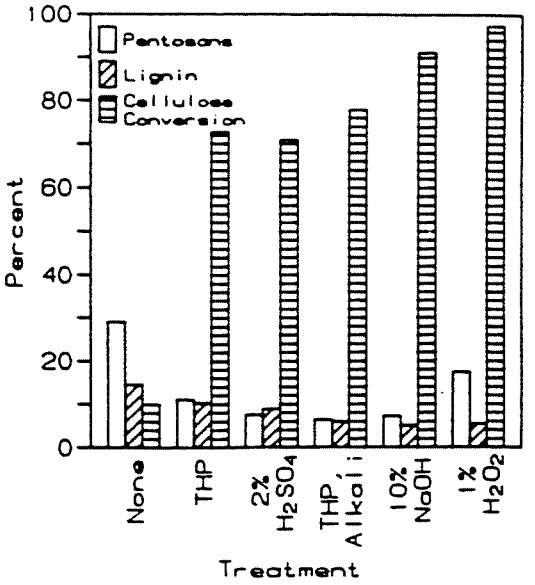

Fig. 1. Relationship of pentosans and lignin in pulps (dry wheat straw basis) to their cellulose conversion by enzymatic hydrolysis. Treatments: THP = thermal hydropulp; water:straw, 7:1, 30 min at 180°C. 2% H_2SO_4 = solution:straw, 7:1, 1 h at 130°C. Alkali = extraction of THP (8% NaOH, dry pulp basis) liquid:solids, 33:1, 30 min at 70°C. 10% NaOH = solution:straw, 10:1, 24 h at room temperature (RT). 1% H_2O_2 = solution:straw, 50:1, 24 h at RT. Shaken at 200 rpm.

CONCLUSIONS

The five treatments using conventional laboratory equipment and procedures to modify WS were successful in obtaining cellulosic-rich substrates that were susceptible to enzymatic attack. An alkaline hydrogen peroxide treatment solubilized and removed sufficient components to permit essentially all of the cellulose in the pulp to be hydrolyzed by cellulase. Even though the pressure

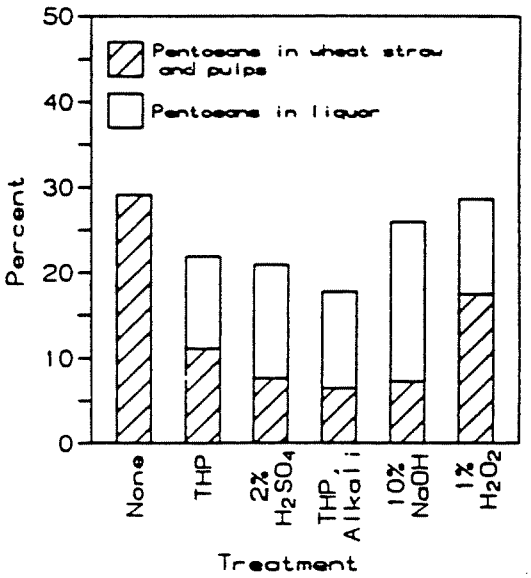

Fig. 2. Pentosans (dry wheat straw basis) retained in pulps and recovered in liquors. Treatments are the same as in Fig. 1.

pulping of WS with water or dilute acid was effective for the removal of pentosans, improvements in the pulping conditions or procedures are necessary to improve the yield of pentosans and/or xylose. Steeping of WS in a 10% NaOH solution and the subsequent precipitation of the pentosans in the liquor with acid and alcohol provided the highest yield of this component. An additional stage or stages should permit a more effective removal and recovery of pentosans and lignin from biomass.

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